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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
MOORE, WILLIAM W	

ART UNIT	PAPER NUMBER
1656	

DATE MAILED: 03/08/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/089,040	UMEZAWA ET AL.	
	Examiner	Art Unit	
	William W. Moore	1656	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *02 December 2005*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,3,6-10,12 and 13* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3,6-10,12 and 13* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Response to Amendment

The Substitute Specification and Abstract filed with the Response of 2 December 2005, and accompanied by the Declaration of the translator, are ACCEPTED. Neither is considered to introduce new matter to the specification where the Substitute Specification corrects and clarifies the great majority of the grammatical and logical inconsistencies previously objected to and also overcomes the objection of record to the specification by removing a sentence added to page 18 of the original specification by the amendment filed 11 March 2005. The Substitute Specification supports the amendments to claims 1, 3, and 6-10, in the Response filed 2 December 2005 as well as new claims 12 and 13 presented therein. The claim amendments and cancellation of claim 11 in the Response overcome the objection of record of claims herein, and overcome the rejections of record of claims herein under the first paragraph of 35 U.S.C. § 112 but require new grounds of rejection of claims 1, 3, 6-8, 10 and the new claims 12 and 13 under the second paragraph of the statute.

Claim Objections

Claims 1, 10, 12 and 13 are objected to because of the following informalities: Claims 1, 10, 12 and 13 are objected to for reciting "half" where the term is misleading because it does not appear in the specification and no division by halves is conceptually disclosed. Claim 12 lacks an appropriate use of the definite article "the" in descriptions at lines 8, 11, and 12 of the claim. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. § 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1, 3, 6-10 and 13 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The claim amendments overcome the rejections of record for indefinite description but the amendments to claims 1 and 10 and the recitations of the new claims 12 and 13 require the following new grounds of rejection. Applicant's arguments filed 2 December 2005 have been fully considered but they are not persuasive. Applicant suggests at page 14 of the Response that the amended claim 1 and the new claim 12 require that each member of a probe set "separately connect" to "target proteins", but claim 1 refers only to "analyzing protein A - protein B interaction" and sites "for connecting" proteins A and B. The orientation of the intein and indicator protein components of the probes "a" and "b" is correct in claim 1, and both page 21 of the original specification and page 16 of the substitute specification provide a literal basis for the term "target", but the term does not appear in the claim and the claim uses the inappropriate term "connected".

Claim 1 is rejected as indefinite because the art recognizes that the amino-proximal and carboxyl-proximal portions of split inteins must be fused – joined by a peptide bond – to the portions of surrounding polypeptide which is ligated to form an integral polypeptide upon excision of the intein portions. Although either probe is properly described without its comprising a target protein, both page 13 of the Substitute Specification and page 21 of the original specification teach that, in a first probe, the N-terminal portion of an indicator protein is fused to N-terminal portion of an intein polypeptide and that, in a second probe, the C-terminal portion of an indicator protein is fused to the C-terminus of the C-terminal portion of an intein polypeptide. The specification further teaches that the C-terminus of the intein polypeptide of the first probe is available for fusion with a first target protein while the N-terminus of the intein polypeptide of the other probe is available for fusion with another target protein. Amending claim 1 to describe this structural relationship, which is essential for proper operation of the probes because "connections" that do not provide fusion polypeptides

in both probes cannot permit excision of their intein portions, will overcome this aspect of the rejection, which affects claims 3, 6-10 because they depend from claim 1 but do not correct its indefinite descriptions.

Claim 10 is independently rejected as indefinite because, in addition to using the inappropriate term "connecting", it fails to describe the location of fusion of protein A, the first target protein, with a particular component of probe "a" and also fails to describe the location of fusion of protein B, another target protein, with a particular component of "b". Claim 10 is further indefinite in failing to describe the nature of a "system" of the second clause of the claim. The specification teaches two systems, an *in vitro* system and an intracellular system, but does not teach, e.g., a mechanical or informational system, and the claim describes an incomplete process where it does not require a system to provide conditions permitting excision of the intein portions of probes "a" and "b" upon interaction of proteins A and B, thus allowing formation of the integral indicator protein so that a change in its signal can be detected

Claim 13 is independently rejected because (1) the claim preamble provides no antecedent basis for the term "the eukaryotic cell" at line 6 of the claim, (2) the claim fails to describe the locations where the protein A-encoding and protein B-encoding polynucleotides are linked to the probe a-encoding and probe b-encoding polynucleotides already present in the expression vector, and (3) is incomplete in failing to provide for conditions suitable for expression of the two fusion polypeptides that comprise, on the one hand, protein A fused to the carboxyl terminus of probe "a" and, on the other hand, protein B fused to the amino-terminus of probe "b" by the host cell.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. § 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject

matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 6, 10, 12 and 13 are rejected, essentially for reasons of record, under 35 U.S.C. § 103(a) as being unpatentable over Remy et al., 1999, and Wu et al., 1998a, in view of Wu et al. 1998b, all of record.

Applicant's arguments filed 2 December 2005 have been fully considered but they are not persuasive. Applicant suggests that the claims exclude indicator polypeptides that are enzymes, i.e., "use various substrates", but claims 1, 6, 10, 12 and 13 embrace all classes of indicator protein and permit the measurement of the change of any kind of signal. Claims 1, 6, 10, 12 and 13 do not require that a protein that serves as an indicator of protein-protein interaction be a fluorescent protein that is not an enzyme or that a claimed method utilize such a probe. Applicant also suggests that the cited prior art does not teach that a signal-generating protein can be formed by intramolecular ligation upon the excision of the components of a split intein but declines to address the teachings of Wu et al., 1998a, and Wu et al. 1998b.

Remy et al. teach the preparation of a bipartite, enzymatic, probe for detection and quantitation of protein-protein interaction wherein fragments of an enzyme are each separately fused to target proteins having a presumed interaction that can be detected, thus forming fusion polypeptides encoded by separate coding regions comprising a split operon within a polynucleotide suitable for common expression of the fusion polypeptides, hence their coexistence, in an eukaryotic host cell. See pages 5394, 5395, 5398 and 5399. Remy et al. further teach that the different regions of the divided enzyme fused separately to each target protein come together to reconstitute a measurable enzymatic activity – a signal – if the target proteins present in the fusion polypeptides interact, and that this permits detection of the interaction of the target proteins by complementing a lack of the enzyme's activity in the host cells that permits cell survival as well as a method for the quantitation of the extent of binding by

fluorescence microscopy spectroscopy wherein a change in fluorescence intensity resulting from protein-protein is measured where one substrate bound by their selected indicator enzyme provides fluorescence when bound by the reconstituted enzyme. See pages 5395-5398 and Figures 1-5. Remy et al. teach, page 5399, that "the design of this system begins with the dissection of a small monomeric enzyme of known structure" and that "because the enzyme is monomeric and results from association-folding of two fragments . . . an observed response is caused by a binary protein-protein interaction. Because Remy et al. do not teach that portions of a split intein should be introduced into their fusion polypeptides between the target protein portions and the separate enzyme portions the teachings of Wu et al., 1998a, and Wu et al., 1998b, were combined with the teachings of Remy et al. in the rejection of record.

Wu et al., 1998a, teach that the naturally-occurring *Synechocystis sp.* DnaE intein has, see Figures 1, 2B, 3A and 3B, an amino terminal portion and a carboxyl terminal portion that are each separately fused to separate portions of the DnaE protein in separate genes widely separated in the cyanobacterium's genome, that this protein is a component of a DNA polymerase essential for DNA replication in the cyanobacterium's cells, and that the intein's amino terminal and carboxyl terminal portions will trans-splice the enzyme component upon recombinant expression from a polynucleotide comprising a split operon in a transformed host cell. Wu et al., 1998b, teach that trans-splicing mediated by amino terminal and carboxyl terminal portions of another cyanobacterial intein, the DnaB intein, that has been split into the two portions will produce a covalent bond joining polypeptides other than the native DnaB helicase when the heterologous proteins are fused to, respectively, the amino terminus of the amino terminal intein portion and the carboxyl terminus of the carboxyl terminal intein portion and the

separate fusion polypeptides are recombinantly expressed from a polynucleotide comprising a split operon in a transformed host cell. See Figures 2 and 3.

The rejection of record is maintained because it would have been obvious to one of ordinary skill in the art at the time the invention was made to prepare a probe for analyzing protein-protein interaction between two proteins by preparing polynucleotide encoding two fusion polypeptides, each comprising a separate fragment of a divided protein that is capable of fluorescence wherein one fragment is fused to an amino terminal portion or a split intein and the other fragment is fused to a carboxyl terminal portion of a split intein and wherein each portion of the split intein is in turn fused to either of two proteins the interaction of which is to be analyzed upon expression of the two fusion polypeptides in an eukaryotic host cell. This is particularly the case where Remy et al. teach that a bipartite, fluorescent indicator protein serving as a probe for the detection and quantitation of protein-protein interaction can be split into fragments that are each separately, recombinantly, fused to target proteins having a presumed interaction to be analyzed and that the separate portions of an indicator protein that is an enzyme will provide a fluorescent signal when brought into close proximity by interaction of the target proteins to reconstitute its activity and where Wu et al., 1998a, teach that an enzyme split into two separate portions can be ligated to reconstitute activity by a split DnaE intein, the amino terminal and carboxyl terminal portions of which are fused to either portion of the split enzyme, wherein the split DnaE intein will splice the separate portions into a free, integral, enzyme.

Such an artisan would have had a reasonable expectation of success in combining either portion of the split enzyme and either of the target proteins to either portion of the split DnaE intein because Wu et al., 1998b, demonstrate that a split intein will splice heterologous fusion partners into a free, integral, polypeptide. Such an artisan would

also have appreciated that intein-mediated splicing to reconstitute the fluorescence of an indicator protein would be effective in providing a fluorescent signal for analysis of protein-protein interaction, see Wu et al., 1998b, to assure proper association of an indicator protein and because a spliced, free, indicator protein would persist in the cell in which it's component portions had been expressed in separate fusion polypeptides.

Conclusion

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL.** See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to William W. Moore whose telephone number is 571.272.0933 and whose FAX number is 571.273.0933. The examiner can normally be reached Monday through Friday between 9:00AM and 5:30PM EST. If attempts to reach the examiner by telephone are unsuccessful, the examiner's Supervisory Primary Examiner, Dr. Kathleen Kerr, can be reached at 571.272.0931. The official FAX number for all communications for the organization where this application or proceeding is assigned is 571.273.8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 571.272.1600.

William W. Moore
3 March 2006

NASHAAT T. NASHED PHD.
PRIMARY EXAMINER